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# Verticillium wilt of alfalfa



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## Verticillium wilt of alfalfa

T. G. ATKINSON Agriculture Canada Research Station, Lethbridge, Alberta M. R. HANNA Agriculture Canada Research Station, Lethbridge, Alberta A. M. HARVEY Alberta Agriculture, Agriculture Center, Lethbridge, Alberta R. J. HOWARD Alberta Horticultural Research Center, Brooks, Alberta H. C. HUANG Agriculture Canada Research Station, Lethbridge, Alberta J. P. MISKA Agriculture Canada Research Station, Lethbridge, Alberta J. W. SHEPPARD Laboratory Services Division, Food Production and Inspection Branch,

Agriculture Canada, Ottawa, Ontario

Summerland, British Columbia

Lethbridge Research Station Contribution No. 1

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J. M. YORSTON

#### COPIES OF THIS PUBLICATION ARE AVAILABLE FROM:

#### British Columbia

Dr. J. M. Yorston
British Columbia Ministry of
 Agriculture and Food
P. O. Box 198
SUMMERLAND, British Columbia VOH 120

#### Alberta

Dr. I. R. Evans
Crop Protection and
Pest Control Branch
Alberta Agriculture
605 Agriculture Building
9718 - 107 Street
EDMONTON, Alberta T5K 2C8

Dr. H. C. Huang Research Station Research Branch, Agriculture Canada LETHBRIDGE, Alberta TlJ 4Bl

#### Saskatchewan

Dr. J. A. Frowd Saskatchewan Agriculture 133 Walter Scott Building REGINA, Saskatchewan S4S 0Bl

#### Manitoba

Dr. G. Platford
Manitoba Department of Agriculture
Agricultural Services Complex
University of Manitoba
WINNIPEG, Manitoba R3T 2N2

#### Ontario

Dr. P. K. Basu Research Station Research Branch, Agriculture Canada 4017C, K. W. Neatby Building OTTAWA, Ontario KlA OC6

#### Québec

Dr. C. Richard Station de Recherches Agriculture Canada 2560 boul. Hochelaga SAINTE-FOY, Québec G1V 2J3

#### Atlantic Provinces

Dr. C. B. Willis
Research Station
Research Branch, Agriculture Canada
P. O. Box 1210
CHARLOTTETOWN, Prince Edward Island
Cla 7M8

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#### **PREFACE**

The recent introduction of verticillium wilt of alfalfa into various regions of Canada calls for vigorous efforts to control this potentially destructive disease and to prevent its further spread.

This technical bulletin on the nature and control of verticillium wilt is designed primarily to assist plant pathologists and extension specialists in providing advice to growers and others involved in various aspects of alfalfa production, processing, or marketing. Each section of the publication has been authored by different contributors from various federal and provincial agencies. Their cooperation in preparing this bulletin on verticillium wilt of alfalfa is gratefully acknowledged.

We trust that the background information and control recommendations provided will be helpful in coping with the challenge of controlling and preventing the further spread of verticillium wilt. The information presented is the most up-to-date available at the time of printing and is the basis for the various control measures recommended. However, since the disease is new to North America and the subject of considerable on-going research, new and more effective measures for combatting verticillium wilt may become available in the future. For this reason, producers and others concerned with the disease should be in regular contact with provincial agricultural extension agencies who will be informed promptly of any significant changes in the control recommendations provided here. We expect that users of this bulletin on the disease will identify changes in content and organization that could improve future printings. Your comments and suggestions are therefore solicited. A questionnaire at the end of the publication is provided for your convenience.

H. C. Huang
T. G. Atkinson
Editors

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#### SUMMARY

Verticillium wilt of alfalfa, a disease new to North America, has become a serious threat to the production of Canada's most important forage legume. This disease is highly destructive, dramatically reducing yields and the productive life of alfalfa shortening stands. Potential losses to hay and forage are estimated to be more than \$250 million because of the importance of this crop to beef and dairy production in all parts of the country. At risk, too, are Canada's expanding alfalfa-seed industry and the dehydrated alfalfa industry, which export more than \$25 million in products annually. technical bulletin outlines the current distribution of the disease in Canada, and how to recognize it, and describes the precautions and control measures that must be applied to prevent further spread of verticillium wilt. It will aid extension specialists and disease experts in advising alfalfa producers and others in the alfalfa industry on how to cope with this new disease threat.

#### Résumé

La flétrissure verticillienne de la luzerne, une maladie nouvelle en Amérique du Nord, est devenue une sérieuse menace pour la production des plantes fourragères au Canada. En effet, cette maladie ravage les cultures en réduisant de beaucoup les rendements et en raccourcissant la période productive des peuplements. On estime à plus de 250\$ millions les pertes potentielles de foin et de fourrage à cause de l'importance de cette culture pour la production laitière et de boeuf de boucherie dans toutes les région du pays. Les industries de la luzerne déshydratée et des semis de luzerne, en expansion présentement, qui exportent pour plus de 25\$ millions de produits chaque année, sont aussi affectées par cette maladie. Ce bulletin technique explique comment est répartie actuellement cette maladie au Canada, comment la reconnaître et il décrit les précautions et les moyens de lutte que l'on peut prendre pour empêcher la flétrissure verticillienne de se répandre davantage. Nous espérons que cette publication aidera les spécialistes de la vulgarisation et tous ceux qui travaillent dans l'industrie de la luzerne à lutter contre cette nouvelle maladie.

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#### 1. WHAT IS THE PROBLEM?

T. G. Atkinson Agriculture Canada Research Station, Lethbridge, Alberta

Verticillium wilt, a destructive disease of alfalfa, has recently been introduced into Canada. It poses a threat to the production of this important forage crop in all areas of the country.

Long-recognized as the most important disease of alfalfa in north-temperate areas of Europe, verticillium wilt was found in 1977 in the southern interior of British Columbia. A year earlier it had been identified in the neighboring State of Washington where the disease appears to have first become established in North America. Since then, it has been reported from most of the states adjacent to Canada.

Verticillium wilt weakens alfalfa stands and spreads so rapidly that yields can be reduced by up to 50% within 3 years. Invariably, the productive life of stands is shortened to 3 years from the customary 6 or more years. The recent experience of alfalfa growers in the interior of British Columbia and in the Pacific Northwest States confirms the reputation of verticillium wilt as being a very destructive disease.

Nation-wide surveys in 1980 and 1981 confirmed that verticillium wilt was widespread in the interior of British Columbia and identified small foci of wilt-infected alfalfa in southern Alberta, southern Saskatchewan, southern Ontario, and Nova Scotia. Although verticillium wilt has not yet been reported in the other provinces, its present distribution indicates that the disease could become established in all areas of Canada where alfalfa is grown. Nevertheless, the fact that the disease is presently widespread only in British Columbia offers a good opportunity to prevent its further spread and possibly to eradicate it in those provinces where the infested areas remain small. Indeed, surveys in 1982 indicated that in Saskatchewan the destruction of wilt-infected fields found earlier had apparently prevented spread to other fields. In contrast, verticillium wilt became more widespread in southern Ontario.

This publication is designed to assist plant pathologists, extension specialists, and alfalfa producers in recognizing the symptoms of verticillium wilt and in getting the disease diagnosed. It describes the damage and economic loss the disease can cause and, most importantly, it describes measures to reduce the risk of spreading verticillium wilt to healthy crops and to get rid of the disease from infested fields.

The spread of verticillium wilt can be stopped and the disease can be eradicated from infested fields if control measures are applied rigorously and persistently by producers and if these efforts are supported by others involved in the alfalfa industry. If nothing else, careful attention to the recommended control measures at each step of handling the crop will avoid widespread losses and will gain time until resistant alfalfa cultivars adapted to Canadian growing conditions are available.

#### 2. WHY BE CONCERNED?

A. M. Harvey and T. G. Atkinson Alberta Agriculture, Agriculture Center, Lethbridge, Alberta and Agriculture Canada Research Station, Lethbridge, Alberta

Alfalfa is the most important and widely grown forage legume. It is a critically important source of inexpensive plant protein for beef and dairy enterprises. Losses resulting from verticillium wilt can disrupt the economy of such forage-fed livestock systems and cut the profits of commercial hay and dehydrated alfalfa suppliers. It could jeopardize important export market opportunities for dehy alfalfa, pedigreed seed, and leafcutter bees. Finally, changes in cropping sequences would be required if fields became infested with verticillium wilt, and this would disrupt established soil-improving rotations.

#### The threat to tame hay production

An estimated 2.9 million hectares (7.1 million acres) or 56% of the tame hay in Canada contain alfalfa in mixed or pure stands (Table 1). This percentage is even higher in Ontario and the Prairie Provinces. Of this alfalfa crop, approximately 60% must be considered "at risk" from verticillium wilt since it is grown either under irrigation or in areas where natural moisture is sufficient to favor the development and spread of the disease (Table 1). Pure alfalfa stands, amounting to 762,000 hectares (1.9 million acres), are at greatest risk. The magnitude and widespread nature of this disease threat pose serious economic implications for the general agricultural economy and individual farms throughout the country.

TABLE 1. ALFALFA PRODUCTION AND ESTIMATED AREAS OF CROP AT RISK FROM VERTICILLIUM WILT IN EACH PROVINCE

	Tame Hay (x 1000 hectares)			
			Wilt Ris	k Zone**
		Containing	pure	
Province	Total*	Alfalfa*	alfalfa	mixed
NOVA SCOTIA	71	7	_	7
NEW BRUNSWICK	70	. 6	_	6
PRINCE EDWARD ISLAND	51	7	-	7
QUEBEC	967	165	-	165
ONTARIO	1,024	550	320	180
MANITOBA	506	450	160	170
SASKATCHEWAN	760	530	130	130
ALBERTA	1,416	1,062	130	240
BRITISH COLUMBIA	290	116	22	48
TOTAL	5,155	2,893	762	953

<sup>\*</sup> Statistics Canada 1981 and Provincial Estimates 1982

<sup>\*\*</sup> Provincial Estimates 1981

#### The effect on crop and livestock production

As a perennial legume, the direct value of alfalfa is in its lifetime yield of high protein forage. But in the year of seeding, alfalfa incurs a cost conservatively estimated at \$300 to \$370 per hectare with little or no return because it establishes slowly. Such costs represent an "overhead" to be deducted from the value of subsequent harvests. In areas where verticillium wilt has become established, alfalfa yields often drop dramatically by the end of the second harvest year and fields may have to be taken out of production after the third. Inevitably, production of alfalfa from such weakened and short-lived stands is more expensive than that from fields normally producing vigorously for 5 or 6 years.

Other costs to the farmer include disruption of the rotation, increased labor, reduced income and cash flow from alternative crops, replacement of forage from off-farm sources or with more expensive substitutes, and a loss of dehy acreage contracts. Using the "at risk" area of 1,700,000 hectares and a conservative average annual hay yield of 6.25 tonnes per hectare valued at \$55 per tonne, a \$584 million industry is at stake.

Farming operations built around intensively managed alfalfa, where yields can be up to three times the annual average, have even greater investment in and dependence on the crop because of its close integration with livestock enterprises. Forage production lost because of the disease must be replaced. Although beef cattle are less demanding than dairy cows, balancing of rations with high protein hay and lower protein corn silage or grain cropping by-products is critical to the economy of the beef industry. The inclusion of high protein alfalfa hay and haylage into dairy rations is the key to economic yield and profit in the dairy industry. On a protein basis only, making no allowance for differences in energy content, the cost of protein in alfalfa hay based on 1981 studies is about one-half the cost of purchased vegetable protein. Thus, replacement of alfalfa forage protein lost to verticillium wilt is expensive.

#### Effect on the dehydrated alfalfa industry

The gross value of dehydrated alfalfa products (pellets, cubes, and meal) produced in Canada is \$37 million, 70% of which results from exports. The 300,000 tonnes of annual production is derived from 64,000 hectares of intensively managed alfalfa. Eighty-eight percent of this production is concentrated in Saskatchewan and Alberta (Table 2). However, regardless of location and environment, all such stands must be considered "at risk" with respect to verticillium wilt because the intensive cutting practices and pure stands involved increase the chance of disease establishment and spread.

TABLE 2. ESTIMATED MAJOR AREAS OF IRRIGATED, DEHY, AND ALFALFA SEED PRODUCTION

	Alfalfa Production (x 1000 hectares)		
Province	Irrigated*	Dehy**	Certified Seed***
OUEBEC	_	1.2	_
ONTARIO	_	3.0	
MANITOBA	_	1.5	2.3
SASKATCHEWAN	3.0	27.8	4.0
ALBERTA	85.0	24.3	5.6
BRITISH COLUMBIA	23.0	1.6	-
TOTAL	111.0	59.4	11.9

<sup>\*</sup> Provincial Statistics, 1981

In the British Columbia interior, where the disease has been established for some years, experience has shown that both the producer and the dehy operator suffer. Producers lose yield and stand longevity while the dehy operator is faced with increased transport and labor costs resulting from having to assemble supplies from a wider radius of his plant. Both suffer losses in time and money resulting from the need to clean and disinfest machinery when moving from infested to clean fields.

#### Effect on the pedigreed seed and leafcutter bee industry

Alfalfa seed production and the associated culture of leafcutter bee pollinators is a highly specialized, valuable, and expanding industry. While western Canada alfalfa seed growers have become the world's leading exporters of leafcutter bees, most of their seed is sold domestically. Despite this, Canada is still a net importer of alfalfa seed. Because verticillium wilt has invaded many seed-producing areas in the Pacific Northwest of the United States, Canadian alfalfa seed growers should be able to expand their domestic and export markets by maintaining and capitalizing on their present disease-free status.

#### Other considerations

The value of the nitrogen fixed by alfalfa must also be recognized when considering the impact of verticillium wilt on agricultural production. In mixed stands, alfalfa contributes to the nitrogen requirements of the

<sup>\*\*</sup> F. Boyce, Market Analysis Branch, Alberta Agriculture, February 1983

<sup>\*\*\*</sup> Canadian Seed Growers' Association 1982 Inspected Acreage Data.
Non-certified seed is also grown across the Prairies, but there are
no reliable statistics

associated grasses. In a rotation, alfalfa leaves organic nitrogen residues, reducing the need for fertilizer nitrogen. Recognizing that more than half of the area of alfalfa is in mixed stands and that not all alfalfa fields are managed to provide the full potential of fixed nitrogen, a conservative estimate would be 60 kilograms of nitrogen per hectare. At current prices, this equates to an annual bonus of at least \$110 million fertilizer nitrogen equivalent. Many effectively managed fields will enjoy up to four times this level of benefit. To the extent that verticillium wilt reduces the vigor and longevity of alfalfa, this nitrogen bonus is "at risk".

#### 3. WHAT IS VERTICILLIUM WILT?

J. M. Yorston
British Columbia Ministry of Agriculture and Food
Summerland, British Columbia

Verticillium wilt is a new and potentially devastating disease of alfalfa in Canada. It is caused by the fungus *Verticillium albo-atrum*, which can spread very rapidly under favorable conditions, reducing forage or seed yields and shortening the life of the stand.

This section describes how to recognize verticillium wilt by the symptoms it causes in alfalfa. It also briefly describes the causal fungus, how it spreads, and conditions that favor its spread. Other crops and weeds that can be affected are listed.

Knowing how to recognize verticillium wilt and understanding the factors that influence its spread within and between fields are important first steps in learning how to avoid and control the disease. Control measures are outlined in Section 4.

#### Recognizing verticillium wilt

Symptoms of verticillium wilt are usually most easily recognized on the regrowth following cutting and begin to appear when the alfalfa is 15-20 cm high. Disease identification in over-mature stands is often more difficult because disease-stunted plants are overgrown and there may be an increase in wilt-like symptoms caused by other factors.

The first evidence of the disease is usually a yellow blotchiness of young leaflets near the top of one or more plant stems. Diseased leaflets show V-shaped yellow to brown segments at their tips. Yellow streaks also develop along the midrib and veins. The young leaflets curl upward and inward, twist along the midrib, and droop (Fig. 1).

Later, the leaves of several or all plant stems are affected and older leaves also show a characteristic yellowing and twisting. Most leaves turn from yellow to a bleached tan color (Figs. 2, 3). Moderately and severely diseased plants are conspicuously stunted (Fig. 2). Although all leaves may be near death, the affected stems remain upright and green (Fig. 3). This is one of the most characteristic symptoms of alfalfa verticillium wilt.

The initial regrowth of a diseased plant following first cutting may appear normal until the plant is 15-20 cm high. Later in the season, severely affected plants may have weakened regrowth, and such plants often die out over winter.

#### Symptoms that may be confused with verticillium wilt

Several factors other than the disease can cause wilt-like symptoms. However, each of the following causes of leaf yellowing and plant wilting can usually be distinguished by careful examination of the symptoms.

#### 1) Drought

The symptoms of early drought usually appear as yellowing and drooping of older, lower leaves. Under severe drought stress the whole plant, including the stems, may wilt and droop (Fig. 6). This stem-wilting does not occur in verticillium wilt. Drought can also be separated from wilt by examining the distribution of wilted plants in the field. Drought signs first appear in groups of plants along gravelly knolls or ridges. In contrast, verticillium wilt-affected plants are often surrounded by healthy, green plants.

#### 2) Frost

Frosts are most likely to affect newly developed leaflets in either the spring or late autumn. Damage appears suddenly with all leaves drooping and turning brown quickly. The ends of the stems bend over into a shepherd's crook (Fig. 7). This characteristic easily separates frost injury from verticillium wilt damage. Light frost may touch only the upper leaves, leaving them initially water soaked and then dead.

#### 3) Boron deficiency

In some areas of the country, boron deficiency symptoms may be common under dry conditions. Plants are stunted. Upper leaves turn yellow (Fig. 8) and sometimes develop a reddish color. Lower leaves remain a healthy green. The leaves remain turgid and do not cup and twist as in verticillium wilt. When present, the reddish color of the leaf is also indicative of boron deficiency.

#### 4) Bacterial wilt

This disease, caused by the bacterium Corynebacterium insidiosum, produces symptoms very similar to those of verticillium wilt. However, symptoms of this disease are not common in Canada because recommended varieties are resistant. European varieties are susceptible to bacterial wilt. Cupping and upward curling of leaflets, plant stunting, and leaf yellowing are symptoms of bacterial wilt (Fig. 9). If the taproot is cut through, a yellowish brown ring just inside the surface can often be seen. It is usually necessary to carry out diagnostic tests in the laboratory to separate bacterial wilt from verticillium wilt.

#### 5) Other diseases and disorders

In addition to those mentioned above, several other diseases and disorders may produce some symptoms in alfalfa that resemble verticillium wilt. These include various crown and root rots, alfalfa sickness, herbicide damage, and injury by certain species of foliar-feeding insects.

#### Causal organism

The fungus causing verticillium wilt of alfalfa, *V. albo-atrum*, can readily be isolated from green stems bearing leaves with wilt symptoms. It is less easily isolated from diseased leaves and roots.

Conidiophores are produced from the fungal hyphae in diseased tissue or on agar cultures. They are multi-celled, often with dark-pigmented basal cells (Fig. 4). Each conidiophore bears 2 or 3 rings of lateral branches, which are arranged in whorls (i.e., verticillate type of branches), 1-5 branches per whorl (Fig. 4). Single-celled, elongated spores or conidia (3-6  $\mu m$  long and 2-3  $\mu m$  wide) are produced singly at the tips of the verticillate branches. Sometimes the spores are aggregated in heads at the ends of branches. The fungus also produces a resting mycelium which is dark brown, thick-walled, and multi-celled.

Another fungus, *Verticillium dahliae*, has been isolated occasionally from alfalfa, but it does not produce as severe symptoms as *V. albo-atrum*. *V. albo-atrum* and *V. dahliae* are distinguishable by the following characteristics:

Characteristic	V. albo-atrum	V. dahliae
Conidiophore	Stout, with dark-pigmented basal cells	Small, without pigmented basal cells
Conidia	Large	Small
Resting structure	Dark brown, thick-walled hyphae	Dark microsclerotia

#### Disease cycle

Verticillium wilt is seldom found in alfalfa fields in the year of seeding. Disease incidence remains low at the beginning of the second year, but it can build up to high levels by the end of the season. The disease pattern in the field may give some indication of the source of the infection. When individual diseased plants are scattered throughout the field, the fungal pathogen was probably introduced with contaminated seed or as air-borne spores from a nearby diseased crop. If the diseased plants are concentrated near the entrance to the field and around the edge, the fungus was probably introduced on harvesting equipment.

In the third year after seeding, the incidence of the disease may be low prior to first cut because most plants infected in the previous year do not survive the winter. This results in a thinner stand, but plant losses up to 30% can often be experienced before yields drop. Surviving plants spread out to fill the empty spaces. Disease incidence in the latter part of the season builds up to very high levels and weed

populations begin to increase. In the fourth year after seeding, plant stand is severely reduced following winterkill of diseased plants. There is extensive invasion of weeds, and yield losses may make the stand uneconomic.

The above pattern of disease progression is typical for areas with a high incidence of disease although exceptions have been observed. Unirrigated crops grown under dry conditions may maintain an acceptable plant stand for 5-7 years even with the disease present.

#### Conditions that favor the disease

Verticillium wilt is favored by high moisture and cool temperatures (near 18°C). In areas of low rainfall, the disease is most evident on irrigated land or in low-lying areas with frequent heavy dews and high humidity. However, verticillium wilt is increasingly a problem on dryland alfalfa in the interior of British Columbia in districts where incidence of the disease is high. The disease has also been detected on unirrigated crops in southern Saskatchewan. Wilt can occur in alfalfa grown on soils ranging from alkaline to acid and light- to heavy-texture.

The disease spreads by means of spores (conidia). These spores are produced on diseased plant parts under moist conditions (Fig. 5) and are spread by wind. A limited amount of spread occurs by root contact. Machinery parts such as cutter blades and tractor tires also spread the spores. Cutter blades carry plant debris from diseased fields and wheels cause plant injury, making the plant more susceptible to infection.

The fungus can survive in the soil for several months. Survival of 9 months on dead alfalfa buried to a depth of 30 cm or for 5 months on the soil surface has been observed in Britain. The fungus is not known to survive in the soil for more than a year in the absence of a suitable host.

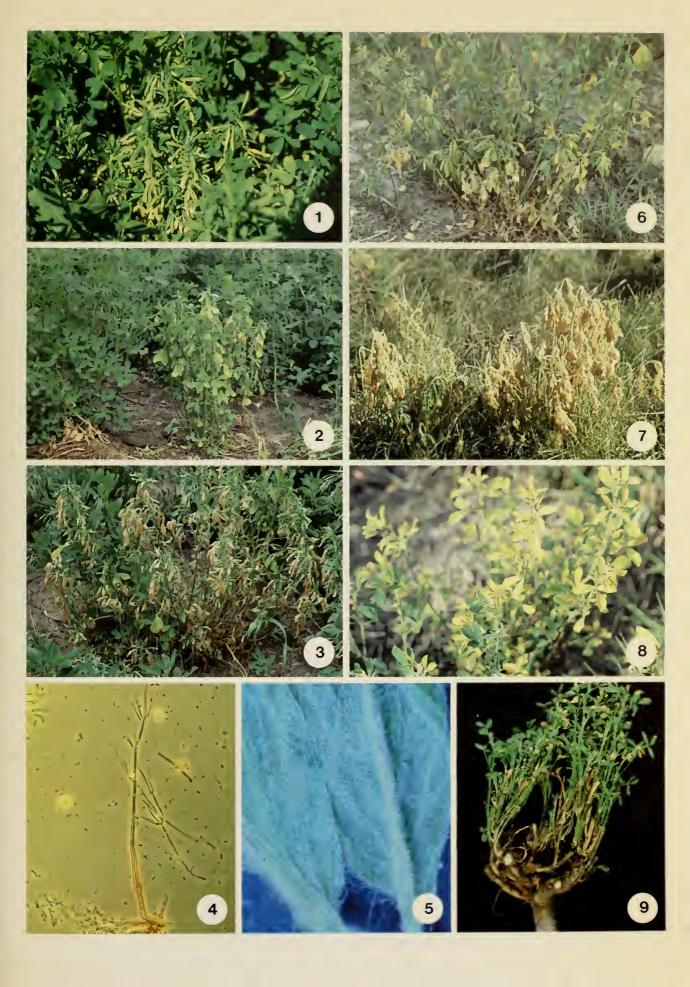
#### Host range

Information on the host range of *V. albo-atrum* is conflicting. This is probably because there appear to be several strains of the fungus. The alfalfa strain is very damaging to alfalfa but has minimal effect on most other crop plants. Pathogenicity tests are required to separate the alfalfa strain of *V. albo-atrum* from strains of the fungus that are major pathogens on other crops.

A wide range of plants harbors the alfalfa strain of the fungus without suffering noticeable damage. These hosts can maintain low levels of the fungus in the soil and thereby carry the disease over to the next crop. Plants that are reported to be hosts of the alfalfa strain of *V. albo-atrum* are: beans, birdsfoot trefoil, canteloupe, eggplant, hairy vetch, hop, lupin, peas, radish, red clover, sainfoin, soybean, strawberry, sweet clover, tomato, watermelon, and white clover. Many common weeds such as Canada thistle, shepherd's purse, plantain, mustard, dock, common groundsel, and yellow rocket are also hosts of *V. albo-atrum*.

#### Legend

- Figs. 1-3. Symptoms of verticillium wilt of alfalfa. Fig. 1, early stage of infection. Note young leaves curl upward and inward, twist along the midrib, and droop; Fig. 2, mid-stage of infection. Leaves are yellow and the plant is stunted; Fig. 3, late stage of infection. Leaves are a bleached tan color but stems remain upright and green.
- Fig. 4 Conidiophores of *Verticillium albo-atrum* with dark-pigmented basal cells. Small, single-celled spores are produced on tips of the lateral branches, which are arranged in whorls.
- Fig. 5 Under moist conditions, spores of *V. albo-atrum* are formed on diseased leaf tissue, producing a grayish white cast.
- Fig. 6 A plant wilted because of drought. Note yellowing of leaves and wilting of stems.
- Fig. 7 An alfalfa plant killed by frost injury. Unlike verticillium wilt, stems bend over in a shepherd's crook.
- Fig. 8 Symptoms of boron deficiency in alfalfa. Note the leaves turn yellow but they do not cup and twist as in verticillium wilt.
- Fig. 9 An alfalfa plant infected with bacterial wilt resulting in leaf yellowing and wilting.





#### 4. HOW CAN VERTICILLIUM WILT BE CONTROLLED?

H. C. Huang and M. R. Hanna Agriculture Canada Research Station, Lethbridge, Alberta

The old saying, "An ounce of prevention is worth a pound of cure", applies well to verticillium wilt of alfalfa. Growers whose fields are free of the disease can prevent the introduction of wilt onto their farms by following preventive measures.

Even when the disease is found in some fields, prompt application of recommended control practices will reduce the risk of the disease spreading and can result in its eradication. The greater the number of growers who consistently apply control measures, the less will be the risk of the disease becoming established. The cost and inconvenience involved in applying these precautions are minor compared with the losses suffered when verticillium wilt becomes established in an area (see Section 2).

#### The disease control strategy

The strategy for dealing with this new threat to Canadian alfalfa production is to:

- a) prevent its introduction into areas now free of the disease;
- b) take prompt and effective measures to eradicate wilt whenever it appears in new locations;
- c) reduce the opportunity for the disease to spread within and between farms if it does become established; and
- d) use wilt-resistant alfalfa cultivars to reduce losses in areas where wilt has become widespread.

The following measures are recommended to eradicate outbreaks of verticillium wilt of alfalfa and to control the spread of the causal fungus. Producers should be aware of the limitations of some of these procedures and incorporate as many safeguards as possible in their operations.

#### Seed treatment

Since the verticillium wilt fungus can be carried with alfalfa seed, precautions must be taken to prevent the introduction of the disease through the use of contaminated seed. A large quantity of pedigreed and commercial alfalfa seed is imported into Canada every year, and some seed lots carry the verticillium wilt fungus. To prevent the introduction of this potentially dangerous pathogen, it is now mandatory that all alfalfa seed (domestic and imported) sold in Canada, except that used for human consumption, must be treated with the fungicide thiram (see Section 6). Seed purchased through the marketplace should already be treated. If it is not, advise your provincial agricultural representative.

Two formulations, Thiram 75 WP (Uniroyal Chemical, PCP No. 15933) and Thiram 320 Flowable (Uniroyal Chemical, PCP No. 16189), are registered for alfalfa seed treatment. Thiram 75 WP is applied as a dust or slurry at the rate of 250 g of active ingredient (341 g of formulated product) per 100 kg of seed. Thiram 320 is applied at the rate of 720 mL per 100 kg of seed. A cement mixer or commercial seed-treater should be used for large quantities of seed to ensure that all seeds are thoroughly coated with the fungicide.

Like other pesticides, thiram is poisonous, so the following precautions are important:

- a) When treating, augering, or handling treated seed, work in a wellventilated area, and wear a suitable dust mask, goggles, and gloves.
- b) Wash hands and exposed parts of the body thoroughly after handling the chemical or treated seed and before eating, drinking, and smoking.
- c) Do not consume alcoholic beverages immediately before or within 24 hours after working with thiram.
- d) Do not contaminate food, feed, or water.

Alfalfa seed should be inoculated with Rhizobium meliloti bacteria prior to planting to encourage good nodulation required for nitrogen fixation. The Rhizobium can be applied by either a sprinkle or slurry method. The Rhizobium container will give details of these methods. Since the seed has been treated with thiram, the slurry method may not be suitable as it will wash off some of the fungicide. A preferred method would be to apply a small amount of sticker (e.g., solutions of Nitracoat, Pelgel, wallpaper glue, gum arabic, powdered milk, or sugar) to the treated seed until it has a glistening (sweating) appearance. The seed should be mixed during application of the sticker to ensure even coverage. Once the sticker has been applied, the inoculant should be gradually sprinkled on until the seed has an even coating. seed will appear black in color. The Rhizobium should not be applied too heavily or a thick crust will form. This may crack and flake-off during handling, thereby resulting in loss of the Rhizobium. inoculation, the seed should be allowed to dry and then planted as soon as possible. The application of Rhizobium as a dry powder without the use of a sticker may not allow sufficient inoculum to adhere to the seed; therefore, this method of inoculation is not recommended for alfalfa. Treatment of alfalfa seed with thiram does not interfere with the Rhizobium inoculant.

#### Field sanitation

A. Elimination of the verticillium fungus carried in fragments of diseased tissue: Debris of verticillium-wilted plants is the main source of infection. Growers should examine their alfalfa for symptoms of verticillium wilt (see Section 3). If an outbreak is the first one on the farm and involves only a few diseased plants or a few isolated patches, efforts should be made to eradicate diseased plants. Since the fungus can spread by direct root contact, healthy plants surrounding each diseased patch should also be destroyed. If only a few infected areas are involved, use of a downward translocated herbicide such as Roundup<sup>®</sup> is probably the most effective means of killing the diseased and adjacent plants. The diseased areas should be clearly marked and inspected periodically to ensure that the disease is checked.

- B. Prevention of spread within and between fields: Machinery movement, air-borne spores, root contact, and irrigation water can contribute to the spread of the verticillium pathogen within or between alfalfa fields. The following measures are recommended to prevent the disease from spreading:
  - a) Precautions to prevent the spread of verticillium wilt by mechanical means-
    - If both diseased and healthy fields are to be harvested, always cut the healthy fields first.
    - To ensure that harvest equipment does not carry infected plant material from one field to another, it should be cleaned and washed with a pressure hose after harvest. This is especially important when moving from a diseased field to a clean field. Disinfestants such as 2% formalin, 5% lysol, or 10% bleach solutions can be used to clean the machinery before leaving an infested field.
  - b) Handling alfalfa hay Because the disease can be carried with the hay, it is advisable that forage from infected crops not be stacked or fed on forage land. Since the fungus is apparently destroyed by ensiling, it may be practical to produce silage rather than hay from diseased crops. See Section 5 for details.
  - c) Irrigating alfalfa fields The moist soil conditions created by irrigation encourages the verticillium fungus in infected tissue to produce spores (Fig. 5) and such spores can be transported from one place to another by flowing water. Growers should not allow irrigation water to flow from diseased to clean fields.
- C. Destroy unproductive, diseased crops: Alfalfa fields with heavy levels of verticillium wilt will be unproductive and should be plowed down.
- D. <u>Control weeds</u>: Many weeds such as shepherd's purse, Canada thistle, and yellow rocket are known to be hosts of *Verticillium albo-atrum*. Because such weeds will allow the long-term survival of the fungus in the field, they should be controlled in fields used in alfalfa rotations.

#### Crop rotation

The verticillium fungus is a poor competitor with other microorganisms that occur in the soil. It soon disappears from the soil if no suitable hosts are present for 2 or 3 years. Rotations of alfalfa with non-host crops such as wheat, barley, corn, oilseed crops, and grasses will be effective in eradicating this fungus from the soil of infested fields.

Since the verticillium fungus may survive in debris from the previous alfalfa crop, a rotation of less than 2 years without alfalfa is insufficient to deplete the pathogen from the soil. An effective rotation is to grow non-host crops for at least 3 years in the infested field. All volunteer alfalfa plants, including those on the headlands and road and railway right-of-ways adjacent to diseased fields, should be controlled. Herbicides can be used to kill volunteer plants by spot treatments.

Alfalfa strains of *V. albo-atrum* may attack soybean, sainfoin, red clover, sweet clover, kidney bean, and peas (see Section 3). These crops should not be used in rotation with alfalfa if verticillium wilt has been a problem.

#### Resistant alfalfa varieties

Resistant varieties usually offer the most effective and economical method of combating plant diseases. Fortunately, genetic resistance to verticillium wilt of alfalfa is available to the plant breeder from various sources.

Verticillium wilt-resistant alfalfas have been developed since about 1965 in a number of European countries. These varieties have been introduced for testing in Canada and the United States, and some have performed fairly well on the basis of the limited results obtained since 1980. One of the major weaknesses of the European varieties is that they are not sufficiently winterhardy for some areas of Canada. Furthermore, they are all highly susceptible to bacterial wilt caused by Corynebacterium insidiosum. At one time, bacterial wilt was a very serious disease of alfalfa in much of North America, but the widespread use of resistant varieties has reduced its importance. However, bacterial wilt would become serious again if susceptible varieties were grown on a wide scale. Thus, European varieties with resistance to verticillium wilt can be of value only as temporary substitutes for adapted North American varieties. Vertus, a Swedish variety with verticillium wilt resistance, was granted a temporary licence in 1982, particularly for use in severely infested areas of British Columbia.

Breeding programs are underway at several locations in Canada and the United States to incorporate verticillium wilt and bacterial wilt resistance in varieties suitable for different areas. In 1981, the first varieties with resistance to both diseases were released in the United States. One of these, Trumpetor, released by Northrup King and Company, was licensed for sale in Canada in 1983.

A breeding program at the Agriculture Canada Research Station, Lethbridge, Alberta, is being carried out to produce alfalfa lines with combined resistance to verticillium wilt and bacterial wilt. From the success attained so far in this and other breeding programs, Canadian farmers in areas where the disease becomes established should soon have the choice of a number of varieties having the disease resistance and agronomic performance required for our climatic conditions.

#### 5. WHAT PRECAUTIONS SHOULD BE TAKEN?

R. J. Howard
Alberta Horticultural Research Center, Brooks, Alberta

Alfalfa infected with verticillium wilt will contain the pathogen in most parts of the top growth. Hence, products derived from infected plants represent a potential source of infection and serve to spread the disease when transported from the field. The risk of such spread can be minimized by following the recommendations given below. Producers should be aware of the limitations of some of these procedures and attempt to incorporate as many safeguards as possible into their operations. The cost of implementing such controls should be offset, in whole or in part, by lower yield losses.

#### Alfalfa hay

The verticillium fungus can survive for several months in cured hay. Livestock producers should therefore avoid stacking or feeding hay from infected alfalfa on fields containing forage legumes, especially alfalfa and clover. It is believed that passage through a healthy animal will destroy the fungus so the manure could be safely spread on fields. However, if animals are diarrhetic or if unconsumed hay becomes mixed with the manure, it should not be used for fertilizer on forage or seed legume crops. When infested hay is being fed, the use of bunkers, troughs, and other types of facilities that restrict livestock feeding to one location is preferred over spreading the feed on the ground. If such facilities are not available, producers should erect temporary corrals for confined feeding or consider having the hay pelleted or ensiled before using it.

Hay producers who know or suspect that verticillium wilt is present in their fields should take steps to reduce the risk of disease spread, especially if the hay is moved off their farms. Loads should be well secured and, if possible, covered to prevent the loss of bales and loose debris during transport. Once unloaded, truck beds should be swept to remove loose hay. Sellers of infested hay should inform buyers that the disease is present so they can take the aforementioned precautions while transporting and feeding the product.

Harvesting machinery suspected of coming in contact with wilt-infected alfalfa should be cleaned and disinfested before moving into a new field. Sanitation procedures for machinery are discussed in Section 4.

#### Dehydrated products

Alfalfa is subjected to high temperatures and drying when processed into pellets and cubes. This heat treatment is believed to destroy most of the verticillium fungus if the dried alfalfa is exposed to 55°C for at least 2 minutes. This temperature is usually exceeded during pelleting but may not be during cubing; hence, the pelleting process is the

preferred way to dehydrate infested alfalfa. If desired, infested hay can be processed before sale or use.

Anyone harvesting dehy alfalfa from fields where verticillium wilt is known to occur should follow the sanitation procedures for equipment discussed in Section 4. Dehy plant operators should clean up spills of unprocessed alfalfa to reduce the risk of infested debris being picked up on the tires of transport vehicles.

#### Alfalfa silage

Little is known about how verticillium wilt affects the quality of alfalfa silage. It is believed that proper ensiling will destroy any verticillium fungus present in the raw alfalfa. The main risk in spreading the disease during ensiling rests with the machinery used to harvest and store the crop. Precautions should be taken to clean and disinfest silage harvesters when moving from field to field (see Section 4).

Raw alfalfa that spills from unloading machinery should be cleaned up once the silo has been filled. Tractors used to compact pit silos should have the tires cleaned and disinfested before they are taken into fields of forage or seed legumes.

#### Leafcutter bee cells

Researchers in Washington State have suggested that the verticillium fungus might be carried on leafcutter bee cells. However, it is not known whether cells represent a significant risk in terms of spreading the wilt disease. At present, the verticillium fungus has not been detected either on western Canadian bee cells or in seed fields. In theory, the following points may influence infestation levels and should be considered when handling cells that may be infested:

- Avoid mixing cells from infested fields with those from healthy fields. Set out uninfested cells whenever possible, especially in fields known to be free of verticillium wilt.
- Collect and destroy leaf pieces and other debris in incubators and overwinter storage areas and during stripping and tumbling operations.

#### Alfalfa seed

The verticillium fungus can be carried as a surface contaminant on alfalfa seed and in debris such as stem and leaf pieces not removed during cleaning. There have been reports that a small percentage of alfalfa seeds harvested from diseased plants can be internally infected with *Verticillium albo-atrum*. Canadian law requires that all alfalfa seed, except that intended for sprout production for human food, must be treated with thiram prior to sale in Canada. The fungicidal seed treatment is discussed in Sections 4 and 6.

#### 6. WHAT REGULATORY MEASURES ARE IN EFFECT?

J. W. Sheppard

Laboratory Services Division, Food Production and Inspection Branch,
Agriculture Canada, Ottawa, Ontario

The causal agent of verticillium wilt of alfalfa, Verticillium alboatrum, can be introduced into wilt-free areas with seed of alfalfa or other parts of infected plants. The following quarantine measures are in effect to prevent the disease from spreading into disease-free areas in this country.

#### Seed treatment

Seed treatment is probably the cheapest means of verticillium wilt prevention. Seed treatment will not control the disease in areas where verticillium wilt is present, but it will prevent the introduction of the disease into wilt-free areas by infested seed.

The fungicide thiram is registered as a seed treatment of alfalfa against verticillium wilt. Regulations established in 1979 require that all alfalfa seed sold in Canada must be treated with this chemical. This requirement applies to both imported and domestic seed. The only exception is alfalfa seed intended for the production of alfalfa sprouts for human food.

#### Hay movement

The verticillium wilt fungus may be present in any parts of diseased plants. Thus, the movement of alfalfa hay infected with verticillium wilt from one region to another may spread the disease. The movement of alfalfa hay from known verticillium wilt areas in the United States and Canada is now controlled by plant quarantine regulations. Hay from wilt areas in the United States can move only into the known infested areas of southern British Columbia. Hay products (e.g., pellets) that have undergone processing that would kill the fungus are exempt from this regulation and may be imported. But cubing and wafering processes do not eliminate the fungus and cubed or wafered hay is not exempt from the regulation. Hay from parts of British Columbia west of the continental divide must be pelleted to eliminate any viable pieces of the fungus before transportation to other areas (see Section 5 for more details).

#### 7. WHERE CAN HELP BE OBTAINED?

Limiting the further spread of verticillium wilt of alfalfa in Canada should be the concern of everyone in the agricultural industry. Early detection of the disease improves the chances of eradicating it. Therefore, farmers or others who suspect that alfalfa may be showing symptoms of verticillium wilt (Section 3) should advise their agricultural representative.

Plant samples should be submitted to the nearest diagnostic center for accurate identification of the problem and advice on control procedures if verticillium wilt is confirmed. Some of the centers that provide disease diagnostic services in various regions of Canada are given in the accompanying list.

To ensure that plants submitted for diagnosis of verticillium wilt are suitable for this purpose when they arrive, follow these shipping instructions:-

- a) send all above-ground parts suspected of having the disease
- b) place the freshly collected materials in paper bags (do not use plastic bags) and ship them promptly, in a suitable mailing container, to the nearest diagnostic center
- c) include the following information:

your name, phone number, and address year crop seeded, date sample collected and submitted distribution of suspect plants (localized or widespread) use of crop (forage, seed, other).

#### List of Diagnostic Centers for Verticillium Wilt of Alfafa in Canada

#### British Columbia

Dr. J. M. Yorston
British Columbia Ministry of
Agriculture and Food
P. O. Box 198
SUMMERLAND, British Columbia VOH 120
Telephone: (604) 494-0401

#### Alberta

Mr. P. Ellis Regional Crops Laboratory Alberta Agriculture P. O. Box 7777 FAIRVIEW, Alberta TOH 1L0 Telephone: (403) 835-2291 Dr. I. R. Evans
Alberta Agriculture
605 Agriculture Building
9718 - 107 Street
EDMONTON, Alberta T5K 2C8
Telephone: (403) 427-5350

Dr. R. J. Howard Regional Crops Laboratory Alberta Agriculture Alberta Horticultural Research Center BROOKS, Alberta TOJ 0J0 Telephone: (403) 362-3391

Dr. H. C. Huang Research Station Research Branch, Agriculture Canada LETHBRIDGE, Alberta TlJ 4Bl Telephone: (403) 327-4561 Dr. P. D. Kharbanda Alberta Environmental Centre Alberta Environment Bag 4000 VEGREVILLE, Alberta TOB 4L0 Telephone: (403) 632-6767

Mr. J. Letal Regional Crops Laboratory P. O. Box 10 OLDS, Alberta TOM 1P0 Telephone: (403) 556-8421

#### Saskatchewan

Dr. J. A. Frowd Saskatchewan Agriculture 133 Walter Scott Building REGINA, Saskatchewan S4S 0Bl Telephone: (306) 565-4671

#### Manitoba

Dr. G. Platford
Manitoba Department of Agriculture
Agricultural Services Complex
University of Manitoba
WINNIPEG, Manitoba R3T 2N2
Telephone: (204) 269-1220

#### Ontario

Dr. L. Busch
Department of Environmental Biology
University of Guelph
GUELPH, Ontario NlG 2Wl
Telephone: (519) 824-4120

Dr. J. W. Sheppard
Food Production and
Inspection Branch
Agriculture Canada
Plant Products Building, No. 22
Carling Avenue
OTTAWA, Ontario KlA OC5
Telephone: (613) 995-4907

#### Québec

Dr. C. Richard Station de Recherches Agriculture Canada 2560 boul. Hochelaga SAINTE-FOY, Québec GlV 2J3 Telephone: (418) 694-4028

Dr. W. E. Sackston Macdonald College McGill University STE. ANNE DE BELLEVUE Québec H9X 1C0 Telephone: (514) 457-2000

#### Atlantic Provinces

Dr. C. B. Willis
Research Station
Research Branch, Agriculture Canada
P. O. Box 1210
CHARLOTTETOWN, P. E. I. ClA 7M8
Telephone: (902) 892-5461

### 8. A SELECTED BIBLIOGRAPHY OF VERTICILLIUM WILT OF ALFALFA AND RELATED CROPS

J. P. Miska and H. C. Huang Agriculture Canada Research Station, Lethbridge, Alberta

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#### 9. Questionnaire

Your suggestions and comments will provide a basis for improving the usefulness of this publication in subsequent printings. Please use the form below and send it to:

> Dr. H. C. Huang Agriculture Canada Research Station Lethbridge, Alberta TlJ 4Bl

Comment	ts on [name of section	(s)]:	
	1		
Any oth	ner comments or sugges	tions?	
Any Oct			
From:			
	Address:		<del></del>
	Type of business:		
	Agribusiness	Farming-dryland	
	Research	Farming-irrigated	
	Extension	Ranching	
	University	Other (identify)	

#### CONVERSION FACTORS

	Approximate	
	conversion	
Metric units	factors	Results in:
LINEAR		
millimetre (mm)	× 0.04	inch
centimetre (cm)	× 0.39	inch
metre (m)	× 3.28	feet
kilometre (km)	× 0.62	mile
AREA		
square centimetre (cm <sup>2</sup> )	× 0.15	anne inch
square metre (m <sup>2</sup> )	× 1.2	square inch
square kilometre (km <sup>2</sup> )	× 0.39	square yards
hectare (ha)	× 2.5	square mile
ricciale (na)	X 2.5	acres
VOLUME		
cubic centimetre (cm <sup>3</sup> )	× 0.06	cubic inch
cubic metre (m <sup>3</sup> )	× 35.31	cubic feet
cubic metre (m <sup>3</sup> )	× 1.31	cubic yards
		January Marad
CAPACITY		
litre (L)	× 0.035	cubic foot
hectolitre (hL)	× 22	gallons
hectolitre (hL)	× 2.5	bushels
WEIGHT		
gram (g)	× 0.04	oz avdp
kilogram (kg)	× 2.2	lb avdp
tonne (t)	× 1.1	short tons
ACRIANI TURAN		
AGRICULTURAL	× 0.000	
litres per hectare (L/ha)	× 0.089	gallons per acre
litres per hectare (L/ha)	× 0.357	quarts per acre
litres per hectare (L/ha)	× 0.71	pints per acre
millilitres per hectare (mL/ha)	× 0.014	fl. oz per acre
tonnes per hectare (t/ha)	× 0.45	tons per acre
kilograms per hectare (kg/ha)	× 0.89	lb per acre
grams per hectare (g/ha)	× 0.014	oz avdp per acre
plants per hectare (plants/ha)	× 0.405	plants per acre



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Verticillium wilt of alfalfa

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